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THE METABOLIC GRADIENTS OF VERTEBRATE EMBRYOS. I. TELEOST EMBRYOS.

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I. INTRODUCTION.

The present paper is a record of observations on the disintegration gradients of some teleost embryos. Since these gradients have served to explain much that was formerly obscure in the physiology and development of organisms, it has seemed to us worth while to study them in as wide a range of living forms, both adult and embryonic, as practicable. In particular it has seemed desirable to determine the susceptibility gradients in the vertebrates in order to analyze their rôle in normal and teratological development and in the physiology of at least certain systems.[1] Since it is impracticable to study adult vertebrates by the susceptibility method owing to the difficulty of determining the time of death of different regions, our attention has been necessarily confined to embryonic stages. The recent publication of Bellamy ('19) on the frog represents the first of these investigations on the vertebrate embryo. In this paper Bellamy described the disintegration gradients of the frog at various stages of development and showed that the teratological forms experimentally produced by him and by a number of previous investigators can be easily and simply accounted for on the basis of these gradients. Similarly in these papers I shall describe the disintegration gradients of other vertebrate embryos and attempt to correlate them with well-known facts of normal and teratological development.

When an organism is exposed to a lethal concentration of a toxic solution, it is found that all of its parts do not die simul-

[1] In particular the gradients have been of great service in interpreting the physiology of the nervous system, the digestive system, and the heart.

taneously, but that they die at different time intervals. In other words, the parts of the organism exhibit a *differential susceptibility* towards the toxic substance. This differential susceptibility bears a direct relation to the organization and axiation of the organism. In the simplest cases this relation is: that the anterior or apical end is the most susceptible and dies and disintegrates first, and that the susceptibility decreases in a graded manner from the apical end along the antero-posterior or apico-basal axis. A death or disintegration gradient (so-called because the time of death is recognized by the disintegration of the part affected) is thus observable. This simple gradient has been designated by us the *primary gradient*. In more complex forms *secondary* gradients often appear after the early stages of development and may persist throughout life; they consist in the appearance of regions of high susceptibility other than the apical or anterior end. In complex animals it is further found that organs and systems may develop gradients of their own which do not necessarily correspond to or bear any relation to the more general primary gradient of the organism. As an example of an organ possessing an axiation independent of that of the rest of the body may be mentioned the vertebrate heart.

The primary gradient is initiated in protoplasm through the action of the external world upon it. Such environmental action is designated a stimulus and the point of stimulation becomes *ipso facto* the region of high susceptibility from which both the effect of the stimulus and the susceptibility gradually diminish, finally dying out. Under repeated stimulation such gradients, originally temporary, become morphologically fixed in the protoplasm to a more or less permanent degree. Since these gradients arose in response to the external world they are retained most completely and in a least altered condition in the external surface of organisms, and its derivative, the nervous system. As the internal organs and systems increase in number and complexity it is not to be expected that they would necessarily develop gradients corresponding to the gradient of the surface structures but rather that, since the gradient is the expression of physiological activity, their gradients will be the expression of their relation to the

organism as a whole and to its parts. Consequently secondary gradients are of common occurrence in complex animals.

Owing to the fact that the disintegration gradients are observable and identical in a wide range of substances of very varied chemical properties and constitutions, it is certain that they are not due to any specific action of these substances upon protoplasm. This is further evidenced by the fact that organisms display the same differential susceptibilities to extremes of physical conditions, such as high temperatures, and to low oxygen supply. Owing to the fact that differential susceptibility is directly related to physiological conditions such as age, starvation, regeneration, motor activity, stimulation, etc., it is further certain that differential susceptibility is not primarily an expression of structural or morphological gradations along the axes of organisms. All of the facts at hand lead us irresistibly to the conclusion that the gradients are physiological in nature, that they are manifestations of a quantitative gradation in function and metabolism along the axes of organisms. We are therefore accustomed to refer to these gradients as metabolic gradients; physiological gradients would possibly be a better term, as Professor Child has recently suggested.[1]

The susceptibiltiy method is thus a method for determining in a general way the relative rates of activity of different parts of the organism. Its results do not necessarily correspond to determinations of total metabolism since usually the susceptibility of only certain parts of the organism can be determined. It is not claimed that it is an accurate measure of metabolic rate, since too many factors enter into differential susceptibility, or that it should supplant methods of directly measuring metabolic rate, such as oxygen consumption, carbon-dioxide production, or determination of other metabolic end products. It has, however, served to reveal facts not discoverable by any other method at present known to us.

[1] These matters are discussed at greater length in a paper by Child now in press in the BIOLOGICAL BULLETIN and in a book "The Origin and Development of the Nervous System," shortly to be published by the University of Chicago Press. I have had the privilege of reading the manuscript of both publications and wish to acknowledge my indebtedness to them for the present argument.

In studying the metabolic gradients by the direct susceptibility method, the observer watches the time of death of different levels of the organism. It is evident that the completeness and accuracy of his observations depend on two factors: first, the ease with which the death point can be recognized, and second, the visibility of various parts of the organisms. In regard to the first matter, it may be said that the death point is usually recognized by certain changes in the appearance of the part under observation; it becomes white and opaque and loose and finally expands into a shapeless mass of granules. In the case of vertebrate embryos, owing to their delicate, almost diaphanous, structure, these changes are not always detectable with certainty, especially in very early stages, and repeated observation has often been necessary in order to make certain of the course of disintegration. As concerns the second matter, it should be perfectly obvious that the gradients can be determined only for those parts of the organism which can be seen clearly; in general these will be the superficial parts. It is usually impossible to observe the death of the entodermal structures; such is the case in the present study. The transparency of embryos, however, permits more extended observations on internal structures than is usually possible with adult organisms.

II. Disintegration Gradients of Fish Embryos.

1. *Material and Method.*—The disintegration gradients were investigated in the embryos of three species of fish—the killifish, *Fundulus heteroclitus,* the cunner, *Tautogolabrus* (*Ctenolabrus*) *adspersus,* and the cod, *Gadus morrhua.* They were obtained at Woods Hole, Mass., the first two species in June and July, and the cod in December, 1919. Sexually mature *Fundulus* and *Tautogolabrus* were obtained through the supply department and were stripped "dry," that is into vessels containing no or very little water. After fertilization had occurred water was added. In this way a high percentage of developing eggs is obtained, as previous investigators have found. The eggs of the cod were obtained from the Bureau of Fisheries at Woods Hole and I am

greatly indebted to the director of the fisheries station for his kindness in supplying me with an abundance of material. Cod eggs in all stages of development were obtainable at any time.

In studying the gradients the eggs were placed in watch glasses which were filled with the toxic solution, and observed under the low power of the compound microscope. Sometimes the watch glasses were filled full and covered with a circular piece of thin glass with the exclusion of air. It was found, however, advisable in most cases to leave the watch glasses uncovered since it was frequently necessary to turn the eggs with a needle in order to bring all parts into view. The eggs of the cunner and the cod are pelagic floating eggs but very soon after being placed in the solution they become opaque and sink to the bottom. Since the eggs in all three species rest upon the bottom of the watch glass it is easy to turn them into any desired position by mean of a needle.

For the disintegration of the embryos of Tautogolabrus, solutions of potassium cyanide in sea-water were employed, in concentrations of 1/100 mol. or stronger. When work was begun on the eggs of *Fundulus* it was immediately discovered that potassium cyanide was useless for the purpose. Apparently the egg membranes of this fish are impermeable to cyanide for the embryos will live and their hearts will continue to beat for very long periods in relatively concentrated cyanide solutions. It was found that anæsthetics and acids would penetrate the eggs of *Fundulus* very readily and stop the heart beat within a few minutes; but in none of these substances could the disintegration gradients be observed for they seemed to coagulate the embryo and it was consequently impossible to determine the time of death of different levels. Finally after fruitless trials of many substances, it occurred to me to try ammonium hydroxide, as it is well-known that this substance penetrates invertebrate eggs readily. It proved to be entirely satisfactory and was thereafter exclusively employed for the study of the gradients of *Fundulus* and the cod. In making ammonium hydroxide solutions several drops of the pure concentrated solution were added to about fifty c.c. of sea-water; the sea-water was then shaken thoroughly and

filtered to remove the abundant precipitate which is always formed on the addition of alkali to sea-water.

The drawings illustrating the distintegration of fish embryos were copied from free-hand sketches made while the embryos were under observation. They do not pretend to be accurate drawings of the embryos but are simple diagrams. The process of disintegration is represented by stippling.

2. *Disintegration of Early Blastoderm Stages.*—The very early cleavage stages were not investigated. The study began at the stage when a small blastoderm is present consisting of a number of cells. At this time in both *Tautogolabrus* and *Fundulus,* the central cells are found to be the most susceptible and from them disintegration extends to the periphery of the blastoderm. Figs. 1 and 2 show the course of disintegration in the egg of *Tautogolabrus* in an early blastoderm stage consisting of about thirty cells. The most central cells become crenated at their margins and eventually resolve into droplets; this process spreads to the periphery. Similar observations were made upon *Fundulus* eggs but they were not entirely satisfactory. In the ammonium hydroxide solutions the blastoderm of *Fundulus* invariably shrinks and arches up on the yolk. After this change has occurred, the central region of the blastoderm bursts open and subsequently disintegrates; later this disintegration extends to the periphery. These changes are illustrated in Figs. 3 and 4. The alkaline solution disturbs the normal tension existing within the blastoderm and between the blastoderm and the yolk; the blastoderm is loosened at its periphery and shrinks into a mass much smaller than normal. This occurrence has rendered it difficult or impossible to determine the course of disintegration in the early stages of *Fundulus.* It is a question whether such changes as can be observed, such as those represented in Figs. 3 and 4, are real expressions of differential susceptibility or whether they may not be due to the alterations of tension just described. In view, however, of the observations on *Tautogolabrus,* about which there cannot be any question, it is reasonable to believe that in *Fundulus* also the central region of the blastoderm is more susceptible than the periphery.

In the case of the cod, however, the state of affairs is the reverse. The earliest stages examined were those consisting of a small blastoderm composed of a considerable number of cells. At this time the periphery is more susceptible than the central portions. This is the case before the germ ring has become visible. The peripheral portion of the blastoderm becomes sharply separated from a central area and disintegrates long before the latter. This condition is illustrated in Fig. 5, the peripheral ring having disintegrated leaving a central disc still intact. In very young blastoderms, the periphery is equally susceptible at all points, as in Fig. 5, but very soon a differential susceptibility appears. One region of the periphery is found in the majority of cases to be more susceptible than any other part of the blastoderm and from this point of high susceptibility the disintegration proceeds in both directions along the periphery. Two stages in the disintegration of such a blastoderm are represented in Figs. 6 and 7. There cannot be any reasonable doubt, in view of the conditions at later stages, that this peripheral area of high susceptibility is the place at which the embryonic shield is to arise. It may therefore be stated that this region is physiologically different from the rest of the blastoderm long before its morphological rôle becomes apparent.

The metabolic conditions during the early blastoderm stages of these three species of fish are therefore of two kinds. In the case of *Tautogolabrus* and *Fundulus,* the central regions are more susceptible while in the cod the peripheral region has the highest susceptibility. There can be little doubt that these differences are the expressions of real differences in the physiology of development of the two classes of eggs. This matter is discussed further below.

3. *Disintegration of Later Stages of the Blastoderm.*—As the blastoderm of *Tautogolabrus* expands over the yolk, the region of high susceptibility comes to lie more posteriorly, that is, in that portion in which the embryo is to appear. The posterior half of the blastoderm is markedly more susceptible than the anterior half, as shown in Fig. 8. This whole region appears as far as could be determined to be equally susceptible throughout; from

this region disintegration extends forward along the margins of the blastoderm. In later stages when the germ ring is approaching the equator of the egg, the region of high susceptibility is shifted still more posteriorly. At this time the observations are somewhat obscured by the occurrence already described for *Fundulus*. The blastoderm, which covers nearly half of the egg, as shown in Fig. 9, breaks loose at its periphery and shrinks and arches up from the yolk, as in Fig. 10. Nevertheless the course of disintegration was followed in a number of individuals. A certain area along the margin of the blastoderm is more susceptible than any other region, as illustrated in Fig. 10; from this area disintegration spreads forwards and laterally as in Fig. 11. There cannot be any doubt that the region of highest susceptibility is the place where the embryonic axis is to arise. In *Tautogolabrus* by the time that the blastoderm has spread nearly half way around the yolk, the eggs float in a tilted position so that half of the blastoderm is on the upper and half on the lower side. The embryo arises in the center of the lower half. It is therefore a simple matter to determine in eggs of the stage depicted in Fig. 9 where the embryo is to arise. The place where this occurs is the region of the high susceptibility shown in Fig. 11.

Observations on the late blastoderm stages of *Fundulus* were impossible owing to the behavior of the blastoderm. It bursts at some point opposite the place where the embryonic shield is forming; it then shrinks rapidly forming a mass about the embryonic shield. In spite of repeated attempts it was impossible to come to any conclusions regarding the susceptibility relations at these stages owing to this shrinkage.

In the eggs of the cod, the germ ring is differentiated at a very early stage. The germ ring is always more susceptible than the central part of the blastoderm and one region is more susceptible than the remainder of its circumference. The disintegration is therefore the same as before the germ ring has become visible and as depicted in Fig. 6 and 7. There can be no reasonable doubt that the point of high susceptibility in the germ ring is the place where the embryonic shield originates. The shield soon

makes its appearance as a slight bulge on the germ ring as shown in Fig. 12. At this time the region of the shield is the most susceptible part of the embryo and from this region disintegration spreads in both directions along the germ ring, as in Figs. 13 and 14. As the embryonic shield grows forward its anterior margin is most susceptible and from this area disintegration extends posteriorly in the shield as shown in Figs. 15 and 16.

4. *Disintegration of the Early Embryonic Axis.*—After the germ ring has advanced half way or more over the yolk, the embryo appears in the center of the embryonic shield. Two faint lines outline its axis. In *Tautogolabrus* and *Fundulus* the embryonic shield is very faintly defined but in the cod is sharply marked out on the blastoderm. In the two first-named species, observations on the disintegration of the early embryonic axis were much obscured by the occurrence of the shrinkage already described. In but one or two cases in each species was the disintegration followed with certainty. In *Tautogolabrus* at the time when the embryonic axis first becomes visible, the course of disintegration is the following; the embryonic axis fades from view and the region in which it lies undergoes disintegration. Fig. 17 represents the normal blastoderm at this time together with the yolk; the embryonic side of the blatoderm is on the under side of the yolk. Fig. 18 shows the same blastoderm much shrunken, the yolk being omitted; the area where the embryonic axis was is seen to be in process of disintegration. As far as could be determined the whole embryonic region is equally susceptible. In *Fundulus* an early stage of the embryo was observed in but one case. In this individual two regions of high susceptibility were observed, one at the posterior end, the other at the anterior end of the embryonic axis. The first region was the more susceptible of the two; from these two points, disintegration proceeded towards the middle of the axis. Three stages in the disintegration of this individual are illustrated in Fig. 19.

Early stages of the cod embryo were observed with ease. Disintegration begins at the anterior end of the embryonic shield and proceeds posteriorly, more rapidly at the margins, as shown in Figs. 20 and 21. In a slightly later stage, the susceptibility

gradient is the same except that a secondary region of high susceptibility is faintly evident at the posterior margin of the shield. This stage is depicted in Figs. 22 to 26.

5. *Disintegration of Later Stages of the Embryo.*—After the embryo had become established its disintegration gradient was observed with ease in all three species and in a great many individuals. In *Tautogolabrus* disintegration begins at the anterior end of the embryo and proceeds posteriorly along the neural tube to its posterior end. An early embryo is depicted in Figs. 27 and 28 and a later one in Figs. 29 to 32. The disintegration gradient remains the same up to the time when the germ ring closes. The eyes are not very highly susceptible but disintegrate at about the time when the disintegration in the neural tube has extended half way back. The neural tube usually separates from the rest of the embryo and becomes arched. The time of death of the somites could not be determined with certainty as they do not seem to undergo disintegration but remain distinct long after the disintegration of the neural tube is completed. After the germ ring has closed a secondary region of high susceptibility appears at the posterior end of the embryo as shown in Figs. 33 and 34. From this time on there is no further change in the disintegration gradients; there are always two regions of high susceptibility, one at each end of the embryo; disintegration proceeds posteriorly along the neural tube and anteriorly to a slight extent from the end of the tail. In no case was the fate of the somites determined.

In *Fundulus* embryos the disintegration gradients are in general the same as in *Tautogolabrus* with certain exceptions. In *Fundulus* the two regions of high susceptibility are present from the earliest observable stages of the embryonic axis. The posterior end of the embryo is the more susceptible and disintegration begins there, and progresses anteriorly. Disintegration then begins at the anterior end of the embryo and progresses posteriorly. In the very earliest stages as in Fig. 19 this anterior disintegration begins at the tip of the neural axis. Very soon, however, the optic bulbs make their appearance. As soon as this has occurred, the optic bulbs are decidedly the most highly susceptible parts of

the anterior end. After they have disintegrated, disintegration begins at the tip of the forebrain and proceeds down the axis, meeting the disintegration progressing forwards from the posterior end in about the region of the hindbrain. An embryo of this stage, at the first appearance of the optic bulbs, is represented in Fig. 35. The first drawing in this figure shows the normal embryo, the germ ring not yet closed and somites not yet formed; the other three drawings give stages in the disintegration. Both neural tube and mesoderm are involved in the disintegration although naturally the fate of the mesoderm is less readily ascertained, owing to its loose structure.

In later stages of *Fundulus* embryos the disintegration gradients remain the same except that the secondary region of high susceptibility at the posterior end gradually decreases in importance. By the time that a number of somites have formed the eyes are the most susceptible region of the embryo. Following the eyes, the forebrain disintegrates and disintegration proceeds posteriorly. The posterior end then begins to disintegrate and disintegration proceeds slightly forwards from this region, meeting the other wave of disintegration near the posterior end of the embryo. The disintegration of an embryo of this age is shown in Fig. 36. The first drawing shows the normal embryo, the other four the course of the disintegration. The shrinkage of the embryo accompanied by a sinuous bending of the neural tube, which always occurs in later embryos in the killing solution, is also illustrated. The fate of the somites could not be observed with certainty and hence they are omitted but the high susceptibility of the segmental plate region is shown in the second drawing of Fig. 36. In still later stages of *Fundulus* embryos, the eyes are no longer more susceptible than the forebrain but both disintegrate about simultaneously. The fate of the somites could not be observed with certainty as they do not disintegrate readily. In earlier stages the somites appear to disintegrate from the posterior end forwards; in later stages when a number of somites have appeared the anterior and posterior somites seemed to be the most susceptible, the middle ones less susceptible.

The latest stages of *Fundulus* which were investigated are de-

picted in Fig. 37. The disintegration gradient is much the same as described in the preceding paragraph except that a region of high susceptibility has developed in the hindbrain where the cerebellum is forming. The end of the tail has by this time become free from the yolk and is elongating; its susceptibility is increased relative to the preceding stage. The tip of the forebrain and the eyes are about equally susceptible and from them disintegration proceeds posteriorly along the axis in both neural tube and mesoderm. The most anterior and posterior somites are more susceptible than the others and from them disintegration proceeds in both directions to the middle of the embryo. Investigation of later stages of *Fundulus* embryos was not feasible as the embryos do not disintegrate as readily as previously. It may be stated, however, that the auditory vesicles and the buds of the pectoral fins were observed to be regions of high susceptibility.

The later stages of the cod embryo resemble those of *Fundulus* except with reference to the eyes. There are always two regions of high susceptibility, the anterior and posterior ends of the embryonic axis. The anterior end commonly precedes in stages before the closure of the germ ring. Disintegration begins at the tip of the forebrain and passes backwards along the brain and eyes; next the germ ring at the posterior end of the embryonic axis disintegrates and this disintegration extends forwards; the two waves of disintegration meet anterior to the middle of the embryo. The eyes are not highly susceptible as in *Fundulus* but about as susceptible as the forebrain. This stage is illustrated in Fig. 38. As the germ ring closes the susceptibility of the posterior end increases and the eyes become temporarily more susceptible than the forebrain, as in the stage depicted in Fig. 39, where the germ ring is on the point of closure. A later stage is illustrated in Fig. 40. The susceptibility of the eyes has decreased but otherwise the disintegration gradient is the same as in the preceding stage. The disintegration of the somites as shown in Figs. 39 and 40 is the same as in *Fundulus,* the disintegration proceeding from each end to the middle. In both Figs. 39 and 40 a region of high susceptibility exists at the level

of the anterior end of the hindbrain; this appears to be related to the development of the auditory vesicles. In later stages evidences of the appearance of a region of high susceptibility associated with the formation of the cerebellum are present.

6. *The Gradient of the Fundulus Heart.*—This investigation was in reality undertaken for the purpose of studying the gradient of the heart. I was convinced from the facts known about the heart that such a gradient must exist. My observations show that such is the case; however, the matter proved more difficult of demonstration than was anticipated owing to the fact that the outer surface of the heart does not readily undergo disintegration. It was only after repeated observation that it was discovered that the disintegration processes occur only in the interior of the heart, the surface outlines remaining intact.

The gradient was studied in the *Fundulus* heart only, the hearts of the other two species of fish being too small for the purpose. The early stages of *Fundulus* proved unfavorable as the yolk sac bulges up around the embryo and conceals the heart from view. In later stages, after the heart beat is established, the disintegration gradient is invariably as follows. Disintegration begins in the wall of the sinus venosus and progresses rapidly along the heart tube to the arterial end of the heart. In this disintegration, the heart wall dissolves or melts away, leaving however, the external outlines intact; this process sweeps rapidly along the heart from the sinus to the bulbus arteriosus. This gradient was observed in numerous cases in hearts in which there was no visible differentiation along the cardiac tube. In later stages after such differentiation has occurred the gradient is more marked and steeper, that is, the disintegration passes more slowly along the heart. After the interior has disintegrated the heart tube expands but the outlines remain intact.

The gradient in the heart is shown not only by the course of the disintegration but also by the order in which the chambers of the heart cease beating in toxic solutions. In younger hearts, the order of cessation of beat is: bulbus and ventricle, auricle, sinus venosus. The sinus continues to beat feebly after the other parts of the heart have stopped contracting. The explana-

tion of this is that the heart beat originates in the sinus venosus, as has long been known, and is transmitted from the sinus in sequence along the heart tube; in order that such transmission shall occur, the original impulse must attain a certain strength. As the sinus is the most susceptible part of the heart, its beat is weakened by the action of the toxic solution and the impulse generated in it becomes too feeble to be transmitted along the length of the heart tube. At first it is able to reach as far as the auricle but not to the ventricle and bulbus, which consequently no longer contract while the auricle still continues to beat; subsequently the beat becomes too feeble to be transmitted as far as the auricle and the sinus remains beating by itself. In later stages of the heart, the auricle may continue to beat after the sinus has ceased; it is probable that as development proceeds, the auricle develops some slight degree of independence and automaticity of its own, as is well known for the auricles of the lower vertebrates, and being less susceptible to toxic agents than the sinus it may continue to contract after the latter has ceased.

The heart is by far the most susceptible part of the *Fundulus* embryo and dies and disintegrates shortly after the embryo is exposed to the ammonia solution. Older hearts are more susceptible than younger ones. This indicates that the metabolic rate of the heart increases during development.

These observations show that there is a gradation in metabolic rate along the heart tube from the sinus to the arterial end of the heart. Such a gradation is in all probability the cause of the sequence of the heart beat. The sequence of the heart beat is generally stated in textbooks of physiology to be due to the fact that the venous end of the heart possesses a more rapid intrinsic rhythm than the other chambers of the heart and consequently "sets the pace" for them. This is really only another way of saying that the venous end of the heart has a higher rate of metabolism than the rest of the heart tube, for how could it contract more rapidly than they if such were not the case? Nevertheless it does not seem to have occurred to physiologists that in such a simple gradation in metabolic rate rests the explanation of the sequence of the beat. It is a familiar physio-

logical fact that the more rapidly an organ is respiring, the more rapidly it gives off carbon-dioxide and consumes oxygen, the more rapidly does it function. A case very similar to the heart is that of the digestive tract, in which Alvarez ('18) has shown that the duodenal end of the intestine has the highest irritability, fastest respiratory rate, most rapid rate of contraction, and greatest susceptibility to drugs, of any part of the intestine and that these factors decrease along the intestine. The matter of the cause and sequence of the heart beat will be discussed more fully in later papers, as the gradient is more easily demonstrable in the chick heart.

7. *General Summary of Observations on the Gradients of Teleost Embryos.*—Before the appearance of the embryonic axis the central region of the blastoderm is more susceptible in *Fundulus* and *Tautogolabrus,* the peripheral region in the cod. It is highly probable that the central region would also be found to be more susceptible in the cod if a sufficiently early stage were investigated; but unfortunately this was not done. It is evident, however, that the high susceptibility of the central region, if ever present in the cod, is lost at a very much earlier stage than in the other two species. In *Tautogolabrus* (*Fundulus* being unfavorable for observations) the region of high susceptibility then gradually shifts from the central to centro-posterior regions and finally to a point on the germ ring where the embryo is to appear. In the cod also the region of high susceptibility becomes limited to the region of the germ ring where the embryonic shield subsequently develops. From this place in both species the region of high susceptibility grows forwards simultaneously with the appearance of the embryonic axis. In the embryos of all three species of fish there are sooner or later two regions of high susceptibility, the anterior and the posterior end, from both of which disintegration extends towards the middle. The posterior region of high susceptibility arises very early in *Fundulus,* later in the cod, and very late in *Tautogolabrus.* This double gradient exists in both nervous and mesodermal structures, but nervous structures are as a rule far more susceptible. A very high susceptibility of the eyes is a marked feature of *Fundulus* embryos.

Regions of high susceptibility also appear in connection with the development of the auditory vesicles and the cerebellum. A gradient exists in the heart (*Fundulus*), the venous end being the most susceptible and the susceptibility gradually decreasing to the arterial end.

III. Relation of the Gradients to Normal Teleost Development.

While it is not my purpose to enter into a detailed account of teleost development it may not be amiss to point out the bearing of the observations recorded in the preceding section on the normal course of development. In discussing this matter it is necessary to bear in mind the fact that teleost development is highly specialized. Embryologists agree in general that the primitive mode of vertebrate development is illustrated by the ganoids and amphibia, forms with total unequal cleavage. The teleost mode of development is derived from some such type and has probably proceeded along different lines in different groups of teleosts.

In the early stages of the blastoderm it was found that in *Fundulus* and *Tautogolabrus* the central cells have a higher rate of activity, as measured by the susceptibility method, than the marginal cells, while in the cod the margin of the blastoderm is more active. These facts indicate that we are dealing here with two different modes of development. In the two first-named species the germ ring and the embryonic shield are poorly differentiated and only faintly visible; in fact I have not represented them in my figures on this account. It is highly probable that in these species the germ ring plays a minor rôle in the formation of the embryo and that the embryo is produced chiefly through the activity of the central region of the blastoderm. This conclusion is supported by the observations and experiments of Morgan ('95) on *Tautogolabrus* and of Sumner ('03) on *Fundulus*. Morgan concluded that the embryo of *Tautogolabrus* is formed largely of material that has never been part of the germ ring. He also showed that the development is not affected if cuts are made in the germ ring at each side of the early embryonic

shield. Sumner concluded that in *Fundulus* the expansion of the blastoderm is centrifugal since when needles are inserted in the sides of the blastoderm the germ ring fails to expand at the points adjacent to the needles and bays in the germ ring consequently appear at those places. Similar conditions seem to be present in the salmon since according to the experiments of Kopsch ('96) the destruction of spots in the germ ring at each side of the earliest stage of the shield does not affect the formation of the embryo. It is evident that this type of development in which the center of the blastoderm is the region of high activity harks back to such a condition as that seen in the ganoids and amphibia in which the animal pole is the region of greatest activity, as evidenced by more rapid rate of division and greater susceptibility to toxic agents (Bellamy on the frog).

In the cod, on the other hand, development proceeds in a different manner. The expansion of the blastoderm over the yolk is here evidently largely due to the activity of the germ ring. In this fish as contrasted with the other two species, the germ ring becomes visible at a very early stage and it and the embryonic shield are very sharply marked off from the rest of the blastoderm. My observations further show that the margin of the blastoderm in the cod is already differentiated as a region of high activity before the germ ring is morphologically distinguishable. The mode of development of the cod by peripheral expansion may be regarded as a specialization from the more primitive type exhibited by *Tautogolabrus* and *Fundulus* and probably represents a short cut in development with omission of the original centrifugal method of growth.

With the expansion of the blastoderm we next observe in *Tautogolabrus,* a shifting of the region of high activity from the central region of the blastoderm to the central-posterior and finally to a definite region of the germ ring where the embryo is to form. It seems to me probable that in these changes in the *Tautogolabrus* blastoderm we have an illustration of the manner in which the centrifugal method of development is transformed into the germ ring type. The conditions in *Tautogolabrus* thus eventually come to be identical with those very early present in

the cod. The cod has evidently omitted the early stages and very soon arrives at the condition in which the posterior median region of the germ ring is the center of activity. There has thus been produced the typical teleost method of development by means of the embryonic shield.

In vertebrates like the frog having a primitive mode of development it is important to note that a region of high activity also develops in the median posterior point of the 'germ ring, that is to say, the dorsal lip of the blastopore (Bellamy, *loc. cit.*). The frog, however, also retains the region of high susceptibility at the animal pole, so that two regions of high activity are constantly present—the animal pole and the dorsal lip of the blastopore. In the teleosts the former region either is not present from the first or first develops and is then lost; but subsequently a new region of high activity develops at this point. It seems therefore that the germ ring type of development is highly specialized and the attempt to interpret the modes of development of other vertebrates in terms of the germ ring is a mistaken effort; but rather germ ring types should be interpreted as modifications of the method illustrated by the amphibia.

After the region of high activity has become limited to a median posterior area of the germ ring, it extends forwards and the embryo appears in its center. That the germ ring is actively concerned in this extension appears improbable since the anterior end of the shield in the cod and all of the shield except the place where it meets the germ ring in the cunner are the regions of high susceptibility. If the forward growth were produced by the germ ring, by a sort of pushing process, then one would expect the posterior end of the shield to exhibit the highest susceptibility. It seems probable that the shield grows forwards through the activity of its anterior end or possibly in some species with the aid of the surrounding cells of the blastoderm. The conditions are evidently variable in different species. According to Kopsch ('96) if the embryonic shield of the salmon embryo is destroyed the germ ring continues to close but no embryo is formed. Here evidently the embryo arises solely from the material of the shield. In *Tautogolabrus* and *Fundulus*, however, the

cells around the shield probably take part. Thus Morgan ('95) found that in *Tautogolabrus* the embryo is formed by a concentration of material towards the center of the shield. Sumner ('03) states that if the embryonic shield of *Fundulus* is destroyed a new embryonic shield is regenerated. My observations also show that in *Tautogolabrus* and the cod the region about the shield takes part in the formation of the embryo since a large area of high susceptibility is present in the early stages, as seen in my Figs. 18 and 20 to 26. While materials around the shield may contribute to the embryo the work of Kopsch, Morgan, and Sumner agrees in denying such a rôle to the germ ring, in the formation of the early embryonic axis. Cuts or injuries in the germ ring at the sides of the shield do not affect the formation of the embryo but this proceeds in practically normal manner.

Although according to these lines of evidence the germ ring plays no rôle or only a very minor one in the early development of the embryo, it sooner or later becomes involved in the formation of the embryo. This is shown in all three species by the appearance of a region of high susceptibility at the posterior end of the embryonic axis. This active region arises very early in *Fundulus* and this fact indicates that the greater part of the *Fundulus* embryo is laid down by this posterior growing region. This was also the conclusion reached by Sumner since he found that piercing this growing region inhibits the formation of the posterior part of the embryo. This region grows backwards adding to the embryo in front of it, exactly as in the case of the primitive streak of the chick, with which it is no doubt homologous. In the cod the posterior growing region arises somewhat later; and in *Tautogolabrus* very late. In the latter species it is evident that very little of the axis of the embryo is due to the activity of this posterior region and that the embryo elongates considerably without it, apparently through growth at its anterior end. This independence of the embryo of *Tautogolabrus* of the germ ring was already noted by Morgan in 1895 since he found that by placing the eggs in diluted sea-water it was possible to retard or prevent the formation of the embryo without affecting the growth or closure of the germ ring. Experiments on other species are

unfortunately lacking except in the case of the salmon, in which according to the experiments of Kopsch, most of the trunk and tail of the embryo are produced through the activity of the portions of the germ ring immediately adjacent to the early embryonic shield. It seems in view of the facts at hand legitimate to draw the conclusion that in different teleost embryos the amount of material contributed to the formation of the embryo by the germ ring is variable. At one extreme are cases like *Tautogolabrus* in which very little material is so contributed and at the other extreme the case of *Fundulus,* where most of the embryo is formed from a growing point in the germ ring. The disagreement among investigators concerning the mode of formation of the teleost embryo is evidently due to the circumstance that they worked with different species. The mode of development in different species is not identical but rather exhibits various degrees of modification from the primitive vertebrate type illustrated by the ganoids and the amphibia to the extremely specialized type in which the germ ring plays the dominant rôle. Owing to this fact generalizations cannot be drawn from the study of a single species and controversies which have arisen in the past concerning the mode of origin of the teleost embryo are without point.

While it may be said that the evidence indicates that the germ ring does add to the embryo in varying degrees in different species, the facts recorded in this paper do not seem to me to support the theory of concrescence. The embryo is not produced by a concrescence of two areas of the germ ring even in cases like the cod where the germ ring plays an important rôle in development; but there is present in the germ ring at the posterior end of the embryonic axis a region of high activity which in some species, like *Fundulus,* is of great importance in the formation of the posterior part of the embryo. Some of the material of the germ ring passes into the embryo but not in the manner required by the concrescence theory; and the anterior end of the embryo is not formed in this way but by an independent region of activity.

The existence in the development of vertebrates—it is now known to occur in the frog and chick as well as in teleosts—of two regions of high activity, one at each end of the axis, is correlated with the fact that vertebrates are segmented animals. This mode of development is common, as far as our investigations go, to all segmented animals. This "double gradient," as we call it, was first discovered in the annelids (Hyman, '16, and Child, '17). It appears in an early stage of development in annelids and persists throughout life in all of them, so far as tested. It also appears, as we have seen, in the vertebrate embryo and persists as long as the posterior end continues to develop and elongate. In the annelids where new segments form continually throughout life, the posterior region of high susceptibility is permanent and never brought under complete control of the anterior end; but in the vertebrates it eventually dies away and segment formation thereupon ceases. The posterior region of activity is therefore correlated with the process of segment formation. There can be little doubt that segments represent incomplete individuals. As Child ('15*a*) has shown, the anterior end of an organismic axis is dominant over a certain length of the axis; beyond this level physiological isolation occurs, the metabolic activity increases, and new individuals arise. A similar process is at the basis of segmentation. The posterior growing region of the embryos of segmented animals has escaped from the control of the anterior end; it is physiologically isolated, develops a high metabolic rate and proceeds to the formation of new but incomplete individuals, that is, segments. This process of segment formation will continue indefinitely if the anterior end fails to regain control of the entire length of the axis as in the annelids but will cease when this occurs as in the vertebrates and probably arthropods. These matters have been discussed at greater length by Child ('17) and Bellamy ('19).

IV. Respiratory Rate During the Development of Fundulus.

I have made some measurements of the rate of oxygen consumption and the rate of carbon-dioxide output during the de-

velopment of *Fundulus heteroclitus*. Similar experiments had previously been performed by Scott and Kellicott ('16), but apparently no report of these experiments beyond the abstract referred to has ever been published. In this abstract it is stated that during the early stages of *Fundulus* up through the formation of the embryo the rate of oxygen consumption is less than 0.10 c.c. per hour per thousand eggs. This statement is in general true; however, it misses the really fundamental point about the respiratory rate during the early stages. I do know why the marked change which occurs at the time that the germ ring is near the equator of the egg should have missed the attention of these investigators; but at least no mention of this is made in their abstract. They noted a marked increase in rate of oxygen consumption when the heart begins to beat; beyond this time there is no marked rise but a general upward trend with a great increase after hatching.

TABLE I.

RATE OF OXYGEN CONSUMPTION OF THE EGGS OF FUNDULUS HETEROCLITUS DURING DEVELOPMENT.

Results given in cubic centimeters of oxygen consumed per 1000 eggs per two hours in exps. 1, 2 and 3; per 3 hrs. in no. 4. Temp. 20° C.

No. of Experiment.....		1	2	3	No. of Experiment.		4
Time Since Fertilization.	State of Embryo.	Oxygen Consumed.			Time Since Fertilization.	State of Embryo.	Oxygen Consumed.
4–6 hrs.....	2–4 cells	0.07	0.09	0.08	2–5 hrs.	to 8 cells	0.04
6–8 hrs.....	32 cells	0.07	0.06	0.07	9–11 hrs. ...	small disk	0.06
9–11 hrs.....	many cells	0.09	0.09	0.07	26–29 hrs. ..	disk ⅓ over yolk	0.19
22–24 hrs.....	large disk	0.09	0.09	0.08			
30–32 hrs.....	far over yolk	0.12	0.13	0.15	34–37 hrs. ..	embryo with eyes	0.21
2 days.....	embryo present	0.14	0.13	0.14			
					2½ days		0.22
3 days.....		0.10	0.10	0.10	3½ days	circulation	0.36
4 days.....	good circulation	0.17	0.18	0.17	4 + days...		0.27
					5½ days		0.25
6 days.....		0.15	0.15	0.14	later		0.30
Later (average)		0.22	0.23	0.24	(average)		

My determinations of the rate of oxygen consumption were made by the same method used by Scott and Kellicott. The carbon-dioxide output was determined by the phenolsulphonthalein method. Forty eggs of approximately the same stage of

development were placed in tubes with the phenolsulphonthalein solution in sea-water. The pH of the sea-water at Woods Hole was found to be 8.2 by comparison with the set of standardized tubes put out by Hynson, Westcott, and Dunning. The length of time required for the forty eggs in a closed tube to turn the indicator from pH 8.2 to pH 7.6 was recorded at different stages of development. This furnishes a rough measure of the relative rate of carbon-dioxide output at successive stages. The temperature was of course kept constant as nearly as practicable throughout such experiments.

The data on the oxygen consumption are given in Table I. Four experiments were run, each consisting of over a thousand eggs mixed from a number of females. Three of these experiments were run simultaneously, the fourth one at a later time.

The experiments recorded in Table I. show that the oxygen consumption remains about the same through the early cleavage although a slight rise probably occurs. By the time, however, that the blastoderm has spread one third or half way over the yolk a marked rise in the rate of oxygen consumption occurs. In experiment 4 this rise was over 200 per cent., less in the other three experiments. From this time on through the establishment of the embryo the rate remains about the same and may even fall again. Thus the formation of the embryo is not a period of increase in the rate of oxygen consumption but rather the time of high respiratory activity is that period when the germ ring is approaching the equator of the egg. This probably corresponds to the time of gastrulation. After the heart has begun to beat the oxygen consumption rises again as also found by Scott and Kellicott. Beyond this time the determinations yielded rather irregular results. I cannot verify the statement of Scott and Kellicott that there is a general upward trend during these later stages; I found a considerable variability in the amount of oxygen consumed; in general it was very little if any higher than the rate at the time the heart had begun to beat vigorously. In the table the average of these later determinations is given. It should be emphasized that the determinations during later stages are probably unreliable owing to the growth of bacteria on the

eggs. Although the eggs were frequently washed, particularly in experiment 4, this source of error was probably present. It tends of course to make the oxygen consumption appear too great. In experiment 4 in which the eggs were thoroughly washed twice daily, the results are probably more reliable than in the other three experiments; and in this experiment no increase was observed in later stages.

These experiments lead us to believe that the rate of oxygen consumption in the development of *Fundulus* is highest at the time when the germ ring is in the neighborhood of the equator, early on the second day of development. It is probably actually highest per unit weight of protoplasm since from that time on the amount of protoplasm increases greatly but the oxygen consumption does not increase in like proportion; in fact, a considerable part of the oxygen consumption after the third day is due to the activity of the heart. As the embryo is continually increasing in size after this time while the oxygen consumption shows relatively little increase we may reasonably conclude that the oxygen consumption of the embryo per unit weight is actually decreasing. In other words, senescence is already in progress.

The study of the carbon dioxide production yielded similar results. The carbon-dioxide production per unit time increased up to the early part of the second day of development after which it fell, rising again in later periods.

This result, that the metabolic activity of the embryo is at its highest point at the period when the germ ring is near the equator, is in harmony with and furnishes an explanation of previously known facts. Child determined the susceptibility of the eggs of *Fundulus* to phenyl urethane ('15*b*, p. 416). He found that the embryos are killed more quickly at this stage than at any other stage. Since susceptibility is, as I have pointed out in the introduction, a measure of metabolic rate, this result of itself shows that the metabolic rate is highest at that period. My experiments confirm this result of Child's and further illustrate the reliability of the susceptibility method as a measure of rate of activity. Various investigators, as Stockard and Kellicott, whose work is considered at greater length later, have noted that

the *Fundulus* embryo is most affected by reagents when the germ ring is near the equator of the eggs and yields the maximum number of teratological forms at this period. Newman ('15) found that in heterogenic fish hybrids development very frequently stops at this stage.

V. Relation of the Gradients to Teratological Development.

The literature on the structure, occurrence, and experimental production of teratological vertebrate embryos has now attained such vast proportions that an adequate review of it would be a huge task. I shall here consider only the experimental production of terata among teleosts. The discussion applies, however, to vertebrate terata in general, since the mode of development is much the same throughout the vertebrates.

Morgan ('95) found that if the developing eggs of *Tautogolabrus* are placed in diluted sea-water, the development of the anterior end of the embryo is commonly inhibited; the medullary folds fail to close and the anterior end remains flattened out on the yolk. In one case the formation of the embryo was completely inhibited but the germ ring continued to develop and close in normal fashion.

The experiments of Stockard ('06, '07, '09, '10) on the production of teratological forms in *Fundulus* are familiar to every one. His first experiments were performed with lithium chloride. In the stronger solutions, the eggs either cease to develop in an early blastoderm stage or else very abnormal embryos are produced with poorly developed anterior and posterior ends, short bodies, and no eyes. The trunk and auditory vesicles are, however, present. In weaker solutions of lithium chloride, the embryos are less abnormal. The expansion of the blastoderm over the yolk may be retarded resulting in spina bifida. The embryos are commonly short with no eyes or defective eyes. The developing eggs were found to be most sensitive to the treatment between 18 and 22 hours after fertilization when the germ ring is near the equator of the egg. In later experiments Stockard found that a number of substances would produce the same effects on the

Fundulus eggs. He used potassium chloride, lithium nitrate and sulphate, calcium chloride, ammonium chloride, and magnesium chloride, alone or in combinations. In solutions of all of these substances embryos were obtained with poorly developed heads and eyes, or with no eyes, with abnormal hearts and defective circulatory systems, with shortened bodies, and open blastopores. In magnesium chloride in particular, fifty per cent. of embryos with various eye defects were obtained. All degrees of approximation of the eyes were noted, to the cyclopean condition. Many one-eyed monsters were obtained, in which one eye was small or defective or wanting. Associated with the approximation of the eyes was often an abnormality of the anterior part of the head resulting in displacement and elongation of the mouth which projected ventrally like a proboscis. The forebrain in these embryos with abnormal eyes may be nearly normal or reduced; it is always reduced when the cyclopean eye is reduced and defective. In the extreme cases, the olfactory pits were fused also. Later Stockard found that similar conditions could be produced by anaesthetics, except that the eye defects were then accompanied by other defects while with magnesium chloride it is possible to produce defective eyes in embryos otherwise nearly normal. The embryos produced in anæsthetics in addition to defective eyes nearly always have narrow and defective brains, abnormal ear vesicles, and defective posterior ends in the form of spina bifida.

Other investigators have obtained similar results. McClendon ('12*a* and *b*) obtained cylclopic *Fundulus* embryos by means of a number of salts, anæsthetics and alkaloids. He states that in nature cyclopic trout embryos arise in water containing an insufficient quantity of oxygen and that he has observed cyclopean smelt embryos which were possibly caused by an excessive carbon-dioxide content. Gee ('16) obtained abnormal *Fundulus* embryos similar to those of Stockard by alcohol and sodium hydroxide. These embryos were characterized by defective heads and eyes, asymmetrical eyes, absence of eyes, shortened bodies, defective circulation, and spina bifida. Gee found that the defects are obtained if the egg is exposed to the solutions before fertilization or shortly after fertilization. Kellicott ('16)

obtained numerous defective forms in *Fundulus* by exposing the eggs to low temperature at various periods after fertilization. The eggs do not develop while in the refrigerator but if removed even after a number of days to room temperature, some of them will develop and numerous abnormalities are produced. Although it is stated by Kellicott that every possible abnormality arises under these conditions, yet perusal of his data show that the abnormalities are in fact limited to certain parts of the embryos. These are: absent or defective head, absent or shortened tail, various abnormalities of the brain and eyes, abnormalities of the heart or circulatory system, abnormalities of wandering cells and their products.[1] It is evident that the terata obtained by Kellicott fall under the same heads as those obtained by Stockard and others. Kellicott noted a marked susceptibility to low temperature at the time when the germ ring is approaching the equator. The low temperature used by Kellicott is more effective than the chemical solutions employed by others since it greatly inhibits the development without at the same time destroying the blastoderm. It is important to note that when such greatly inhibited living masses are restored to room temperature suggestions of organs develop such as "brain fragments, lenses, portions of optic cups, groups of somites, masses of erythrocytes, rhythmically contractile cells arranged either as flat sheets or tubular hearts, scattered pigment cells of the usual types, endothelial cells over the surface of the yolk, fragments of notochordal tissue." Kellicott did not notice the fact that these fragments which develop from eggs retarded in early stages concern exactly the same parts of the embryo in general as fail to develop when the eggs are inhibited at later periods in their development. Loeb ('15) obtained blind *Fundulus* embryos by means of potassium cyanide solutions and low temperatures. One embryo is figured by Loeb which possesses eyes and tail and nothing else. Werber ('16) again obtained the same teratological types with butyric acid and acetone-embryos with defective heads, including brain (forebrain), mouth, and eyes, with approximated olfactory pits,

[1] No observations were made by me on the wandering cells of the yolk sac. It is reasonable to believe, however, that such cells are cells of high physiological activity and hence highly susceptible to toxic agents.

defective auditory vesicles, defective or absent tails. Werber also noted the same fact which as been mentioned in connection with Kellicott's experiments, namely, that in some cases, the parts which are usually inhibited may alone survive, the rest of the embryo having disappeared. Such isolated parts are the anterior end of the embryo and the eyes. In some cases the only differentiated parts of the embryo were a fragment of the brain with an eye attached.

Similar terata can also be produced by hybridization. Such terata in *Fundulus* hybrids were described by Newman ('08, '17) and Loeb ('15). Newman showed that there is a correlation between the rate of development of such hybrids and the degree of abnormality. Those which develop most slowly showed the most pronounced abnormalities. The terata are of the same types as those already described, consisting of defective and inhibited heads, brains and eyes, defective hearts, shortened bodies, as well as types in which the head and eyes alone are present.

It is highly significant to note that similar terata can be obtained by treatment of the sperm alone. Oppermann ('13) obtained them from normal eggs of the salmon fertilized by sperm which had been exposed to radium and mesothorium. The embryos resulting from such fertilizations show all of the typical defects—distortions and marked inhibition of the forebrain and eyes and general anterior end of the body, defects or inhibitions of the tail, spina bifida, some abnormality of the myotomes. Embryos were frequently obtained having neither definite heads nor tails, but only trunks. G. and P. Hertwig ('13) treated the sperm of *Gobius jozo* with methylene blue and methyl green and observed that eggs fertilized by such sperm produce abnormal embryos with defective anterior and posterior ends.

From this consideration of the literature it is obvious that a large variety of agents and conditions produce the same defects in fish embryos. These defects are primarily concerned with the following parts of the embryo: the forebrain, the head in general, the sense organs, especially the eyes, the heart and circulatory system, the tail.

The explanations of these defects have been almost as numer-

ous as the investigators concerned. These explanations have in general proved inadequate and unsatisfactory and fail to account for the facts. The most obvious explanation, proposed at first by Stockard, that the defects are the consequence of a specific action of the chemicals employed upon the embryo, was later abandoned by him and must be regarded as untenable. The fact that a large number of substances and conditions call forth the same defects at once shows that their action must be a very general one and not at all specific. The osmotic pressure of the solutions cannot be the effective factor, since solutions of varying osmotic pressure yield similar results. McClendon's proposal that the solutions alter osmotic conditions in the egg by changing the permeability of the surface cannot be accepted in view of the fact that the same defects are produced by injuring the sperm only and keeping the eggs in normal sea-water. Stockard's final conclusion that the defects are due to a general depression of the eggs by the agents to which it is exposed contains part of the truth but fails to account for the fact that only certain parts of the embryo are affected. Werber believes that the defects are due to a blastolytic destruction or dispersal of the embryo; but outside of the fact that such blastolysis cannot be demonstrated the theory fails like the others to account for the differential action of the effective agents on the embryo. Kellicott sought the explanation in a disturbance of the normal organization of the egg with abnormal arrangements and distributions of the egg materials. This theory likewise does not account for the differential effect on the embryo.

It is perfectly obvious that the outstanding fact which must be taken into consideration is that all of the reagents and conditions affect some parts of the embryo more than they do other parts. These affected parts have already been enumerated. It is quite impossible to account for this except on the assumption that certain parts of the embryo are more susceptible to alterations of conditions than other parts. The necessity for this assumption has been recognized clearly by Stockard, McClendon, and Werber, but it does not seem to have occurred to them that when this assumption is granted no further explanation is neces-

sary. It is of itself the explanation. The defects are due not to the agents used, except in a general way, but to the metabolic conditions in the egg and embryo.

The work of Child and his students upon the susceptibility gradients of organisms has shown that in fact some parts of the organism are more susceptible to external agents than others. *The differential susceptibility required to explain teratological development is then no longer an assumption but a demonstrated fact.* In the sea-urchin ('16) and in annelids ('17) Child showed that the development could be controlled and modified on the basis of the susceptibility gradients and predictable types of terata experimentally produced. A similar demonstration of the relation between the susceptibility gradients and the teratological development was made by Bellamy on the frog.

The relation between the susceptibility gradients and the production of terata is the following: Those parts of the egg or embryo having the highest susceptibility and metabolic rate are the most strongly affected by altered conditions of a depressing nature and the most greatly inhibited by them, providing that the circumstances do not permit of recovery or acclimation. On the other hand if the circumstances do permit of such recovery and acclimation than those same parts which under more severe conditions succumb are able to recover and continue to develop while parts of lower metabolic rate cannot.

In order to apply these conceptions to any particular organism it is first necessary to study the metabolic gradients in that organism. This I have done in the case of the teleost fishes and I have shown that the most susceptible parts are the forebrain, the eyes (particularly in *Fundulus*), the heart, the posterior end, and to a less extent the other sense organs.[1] It will be obvious without

[1] No observations were made on the susceptibility of the olfactory pits but in the frog Bellamy noted that they are regions of high susceptibility. In general it may be said of the sense organs of the head, that the eye is the most susceptible, the ear vesicles next, and the olfactory pits last. It is therefore possible to obtain defective eyes in embryos otherwise fairly normal but defective ear vesicles and approximated olfactory pits occur only in embryos otherwise considerably abnormal. As the matter is not discussed in the text a word may be said here about the cerebellum. The high susceptibility of the

further discussion that these parts of the embryo, shown by me to be the most susceptible to toxic agents, are also the ones showing the most defective development in the experiments which have been quoted. In all of these experiments it is evident that the agents used are inhibiting or depressing agents because as stated by the authors the development of the eggs subjected to them is slower than that of the control. Under such depressing conditions the parts with the highest susceptibility or, in other words, highest metabolic rate, will be inhibited while other parts develop; and this is actually the fact. On the other hand, if the circumstances permit, such parts can recover more readily than others, and these same parts may be found developed while other parts have succumbed. This explains the development of small parts of the embryo described by Kellicott, Werber and others—isolated eyes, hearts, fragments of brain, etc.

The susceptibility gradients therefore furnish a basis for the explanation of teratological development. No other conception which has been advanced does so serve to account for all of the facts. In particular it seems to me impossible on any other basis to explain the production of the same terata in eggs fertilized by injured sperm or by foreign sperm or in cases where the egg is treated before fertilization as in Gee's experiments. In such cases a general lowering of the metabolic rate of the egg as shown by its slower development has occurred and this could produce specific terata only in case certain parts of the embryo require a higher metabolic rate for their expression than others.

The application of the metabolic gradient conception to vertebrate teratology has already been made by several investigators. Werber ('16) recognized its bearing on the teratological *Fundulus* embryos which he produced but failed to grasp the full significance of the conception and failed to see that it rendered his own conception of differential blastolysis superfluous. Newman ('17)

cerebellum is interesting in view of the fact recognized by neurologists that the cerebellum is a supra-segmental structure added on to the brain stem in the course of evolution; and the further fact, discovered by MacArthur and Jones ('17), that the cerebellum respires about as rapidly as the cerebral hemispheres, both respiring more rapidly than other parts of the central nervous system.

clearly saw that "the principles enunciated by Child serve to rationalize the results of heterogenic hybridization." He gave what is for the most part the correct explanation of the terata originating in his hybridization experiments but fell into a number of errors because little was then known about the metabolic gradients in these fish embryos, and he assumed them to be like those of the flatworms.[1] The most complete analysis of vertebrate teratology which has been made is that of Bellamy ('19) on the frog, since in this case both the metabolic gradients and the terata resulting from the differential action of external agents on the eggs are known.

I have now shown in a general way how the terata produced experimentally in teleost embryos can be explained on the basis of the metabolic gradients. Such terata are of two general types, those due to differential susceptibility, in which the parts of highest activity are inhibited and defective, and those due to differential recovery or acclimation, in which the parts of highest activity alone survive. A more detailed discussion seems to be unnecessary in view of the extensive treatment of the matter in the papers of Child, Newman, and Bellamy already cited. I may, however, as an illustration of the application of the susceptibility results to a specific organ take the case of the *Fundulus* eye. It happens that in *Fundulus,* as I have shown, the eyes are very susceptible with reference to other parts of the body, more so than in other species of fish. This indicates that the region from which the eyes arise must be one of very high activity, and as the data of Stockard show this region must be affected before the eyes appear in order that defective eyes result. Consequently inhibition of this region results first in approximated and later in defective eyes. Now since it happens that in *Fundulus* this region is so much more susceptible than in other forms, the occurrence of eye defects in *Fundulus* will also be more common than in other forms and further it is possible to obtain eye defects

[1] In particular the statements made by Newman about the gradient of the heart and circulatory system are quite erroneous. Further the posterior end of fish embryos is a region of high metabolic rate and embryos with defective posterior ends are probably due to direct inhibition and are not recovery types as supposed by Newman.

in embryos otherwise nearly normal, if the inhibiting agent is a rather weak one. This is not possible in other forms; I venture to predict that such a result could not be obtained in Tautogolabrus but that defective eyes in this species would always be associated with marked defects of the brain and other parts of the head. Such is the case in the frog, where cyclopic eyes occur only in markedly microphthalmic embryos. Owing also to the high metabolic rate of the *Fundulus* eye it is possible in this form for the eye to recover and survive when nearly all other parts of the embryo are killed. I also venture to predict that the occurrence of such isolated and solitary eyes in the absence of other parts of the embryo will be found to be rather rare and unusual in other species.[1]

In conclusion I may reiterate that the study of the metabolic gradients such as has been made in this paper furnishes a rational basis for the understanding and interpretation of normal and teratological development. While the particular organism which is to develop from a given egg is determined by the hereditary constitution of that egg, the orderly sequence of development, the spatial relations and proportions of parts, and the general axial organization are controlled by physiological, metabolic differences between different parts of the developing egg. Such physiological differences arise in the final analysis through the action of external conditions on protoplasm. By modifying in a purely non-specific, quantitative manner the metabolic differences at different levels, orderly predictable departures from the normal course of development are obtainable.

VI. Summary.

1. The susceptibility of developing eggs of *Fundulus,* the cunner and the cod to toxic solutions at various stages was studied.

2. In early blastoderms the central region is more susceptible in *Fundulus* and the cunner, the peripheral region in the cod.

[1] Monopthalmia, often observed in *Fundulus* embryos, is simply due to a greater susceptibility of one side than the other; the eye on the more susceptible side is inhibited. In the course of my studies on vertebrate embryos, this asymmetrical susceptibility has frequently been noted although the figures are drawn as if the susceptibility were always bilaterally symmetrical.

3. In late blastoderms, the median posterior region of the germ ring where the embryonic shield is to arise is the most susceptible region.

4. After the formation of the shield, its anterior portion is the most susceptible.

5. After the origin of the embryonic axis the anterior end of the axis is the most susceptible and from this point the susceptibility decreases posteriorly.

6. Sooner or later a secondary region of high susceptibility arises at the posterior end of the embryo. This secondary region arises very early in *Fundulus,* later in the cod, and very late in the cunner.

7. After the origin of the secondary posterior region, the general susceptibility gradient in all three species is a "double" one. Anterior and posterior ends are the points of highest susceptibility and from them the susceptibility decreases in both directions towards the middle. Both ectodermal and mesodermal structures (somites) are involved in the double gradient but the ectodermal structures (neural tube) are in general much more susceptible.

8. The heart is highly susceptible (*Fundulus*). The venous end of the heart is the most susceptible part of it and from it the susceptibility decreases towards the arterial end.

9. Besides the general gradients, specific organs may exhibit high susceptibility. Conspicuous examples of this are the eyes (especially in *Fundulus*), the auditory vesicles, and the cerebellum.

10. The relations of these gradients to normal development are considered. It is pointed out that the embryo arises for the most part from material that does not come from the germ ring but that later the germ ring contributes to the embryo in degrees varying in different species. It is further pointed out that the germ ring type of development is probably a specialization from a method in which the center of the blastoderm played the chief rôle in development. The facts recorded do not support the theory of concrescence.

11. The oxygen consumption and carbon-dioxide production

of developing eggs of *Fundulus heteroclitus* increase up to the time when the germ ring is at the equator of the egg. Subsequently they decrease relative to the amount of protoplasm but show an absolute increase owing to the heart beat and other activity. This period when the respiratory metabolism is greatest is also the period when the eggs are most readily modified by external agents.

12. The relation of the susceptibility data to teratological development is discussed at considerable length. It is shown that those parts of the embryo having the highest susceptibility are those which are most defective in teleost terata and that such differential susceptibility is therefore the explanation of teratological development. It is also shown that these same parts most susceptible under extreme conditions may recover if conditions permit and may develop while the less susceptible parts fail to recover. Recovery forms of terata are thus just opposite in appearance to inhibited types.

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Explanation of Plates.

Plate I.

Figs. 1 and 2. Two stages in the disintegration of an early blastoderm of *Tautogolabrus*, showing greater susceptibility of the central cells.

Figs. 3 and 4. Two stages in the disintegration of an early blastoderm of *Fundulus*, showing rupture and disintegration of the central region.

Fig. 5. Disintegration of an early blastoderm of the cod, showing greater susceptibility of the margin.

Figs. 6 and 7. Disintegration of a later blastoderm of the cod, showing greater susceptibility of one region of the circumference and spread of disintegration in both directions from this region.

Fig. 8. A later blastoderm of *Tautogolabrus*; posterior half of the blastoderm most susceptible.

Figs. 9 to 11. Disintegration of a late blastoderm of *Tautogolabrus*. Fig. 9, the normal blastoderm; Fig. 10, bulging of the blastoderm up from the yolk and disintegration of the central posterior region; Fig. 11, further course of disintegration.

Fig. 12. First appearance of the germinal shield in the cod.

Figs 13 and 14. Disintegration of a stage like Fig. 12. Fig. 13, disintegration of the shield; Fig. 14, spread of the disintegration around the germ ring.

Fig. 15. First stage in the disintegration of a later stage of the embryonic shield of the cod; disintegration beginning at the anterior end of the shield.

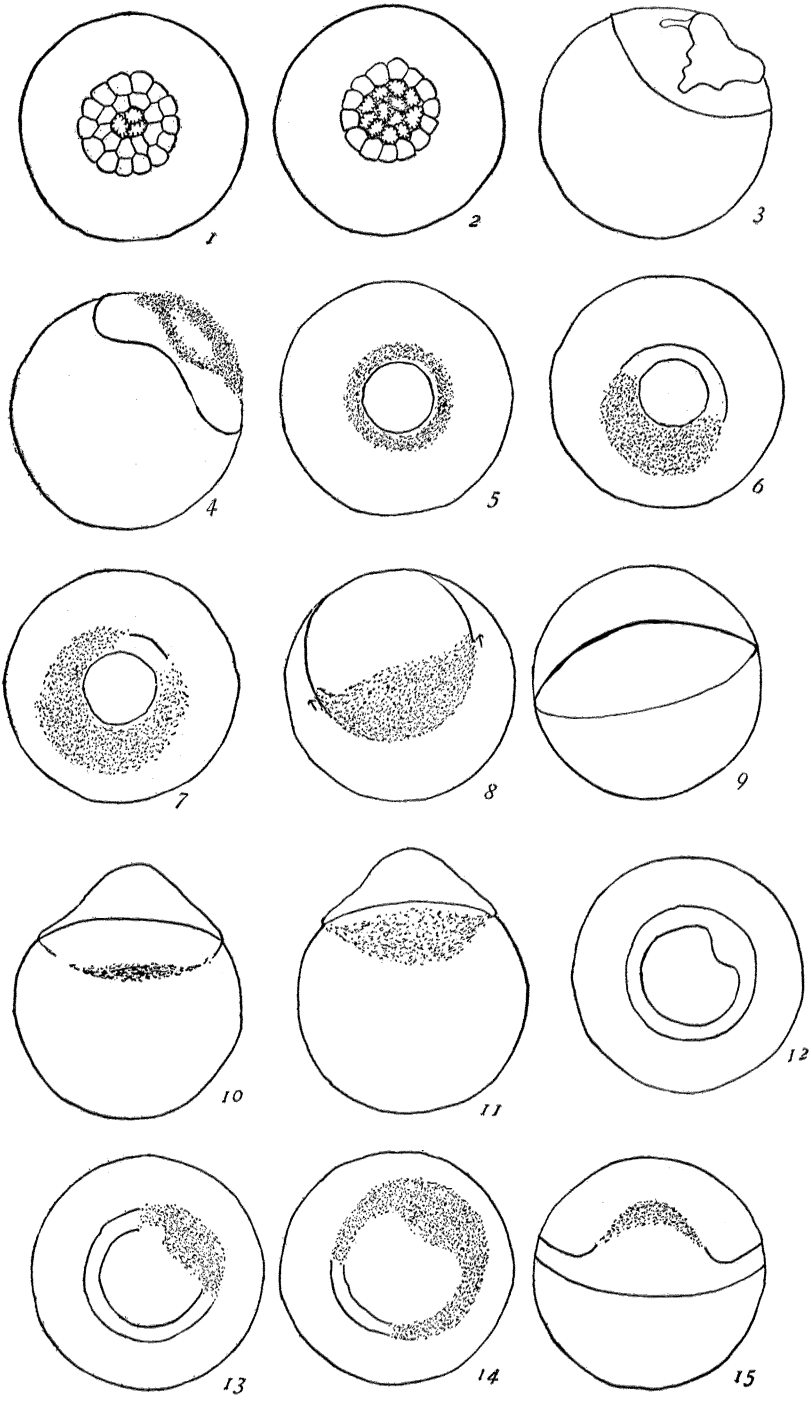

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PLATE II.

FIG. 16. Further course of the disintegration shown in Fig. 15.

FIG. 17. Stage of the early embryonic axis in *Tautogolabrus*.

FIG. 18. Same blastoderm as in Fig. 17, drawn without the yolk; it is much shrunken. The embryonic region is disintegrating.

FIG. 19. Three stages in the disintegration of the earliest observed embryo of *Fundulus*. Anterior end to the left. Disintegration begins at the posterior end, then the anterior end, and proceeds in both directions to the middle.

FIG. 20. Earliest appearance of the embryo in the cod. Disintegration beginning at the anterior end of the embryonic shield.

FIG. 21. Further course of the disintegration shown in Fig. 20.

FIG. 22. Later embryo of the cod; disintegration beginning at the anterior end of the shield.

FIGS. 23–26. Further course of the disintegration shown beginning in Fig. 22.

FIG. 27. An early embryo of *Tautogolabrus*. Disintegration is beginning at the anterior end of the head.

FIG. 28. Same embryo as Fig. 27, enlarged, showing course of the disintegration along the neural tube.

FIG. 29. An embryo of *Tautogolabrus* shortly before the closure of the germ ring.

FIGS. 30–32. Three stages in the disintegration of the embryo shown in Fig. 29.

FIG. 33. Normal embryo of *Tautogolabrus* after the closure of the germ ring.

FIG. 34. Three stages in the disintegration of the embryo shown in Fig. 33. The somites are omitted in the first two drawings.

FIG. 35. An embryo of *Fundulus* after the appearance of the optic vesicles and three stages in its disintegration.

FIG. 36. A later embryo of *Fundulus* and four stages in its disintegration. The somites are omitted from the latter. The neural tube is characteristically curved.

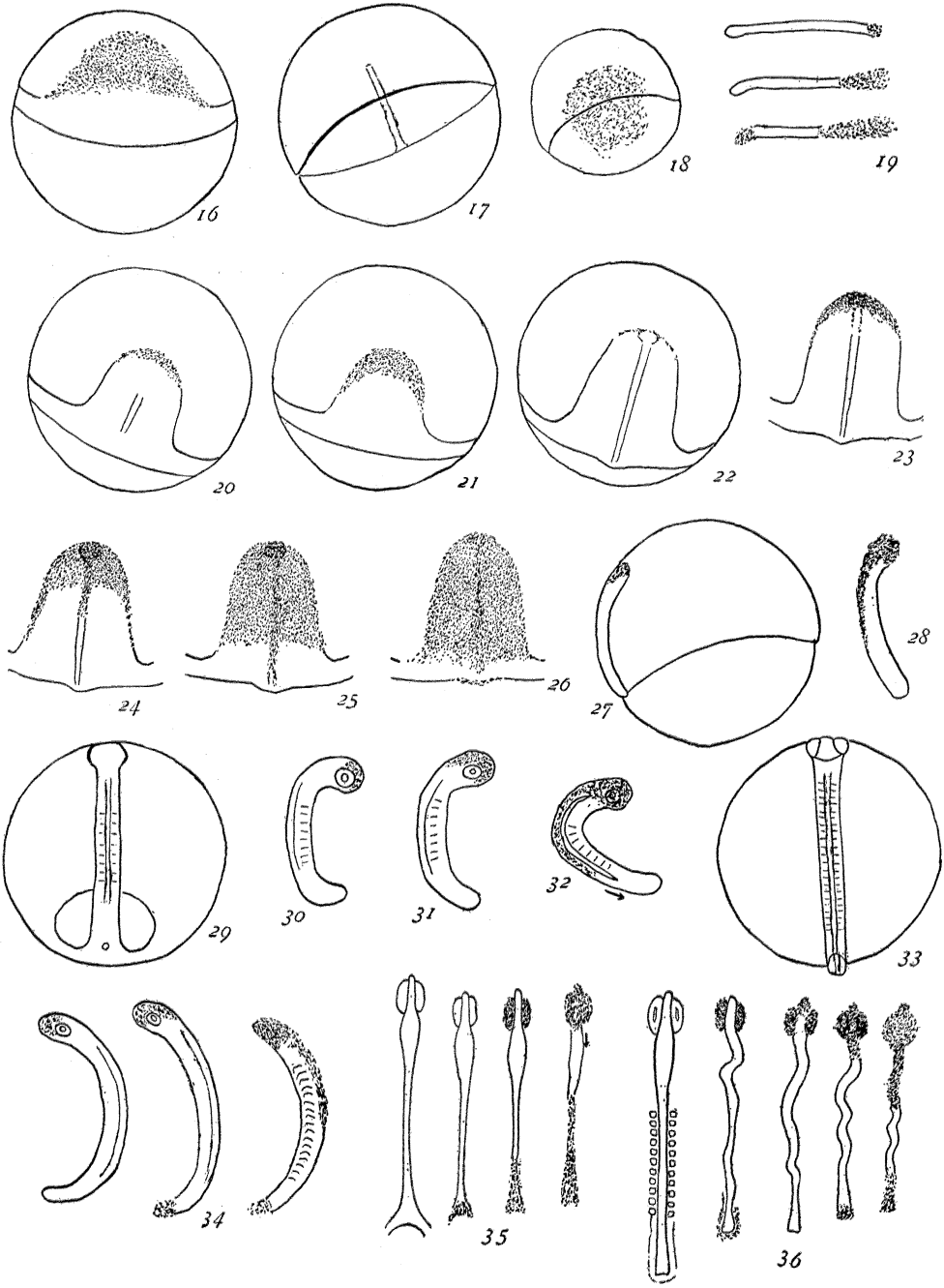

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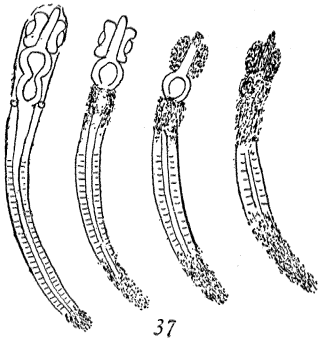
PLATE III.

FIG. 37. Four stages in the disintegration of a later embryo of *Fundulus*.

FIG. 38. Three stages in the disintegration of an embryo of the cod after the formation of the optic vesicles.

FIG. 39. Four stages in the disintegration of an embryo of the cod shortly before the closure of the germ ring.

FIG. 40. Four stages in the disintegration of an embryo of the cod after the closure of the germ ring.



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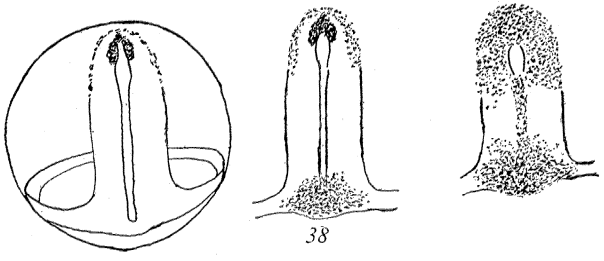

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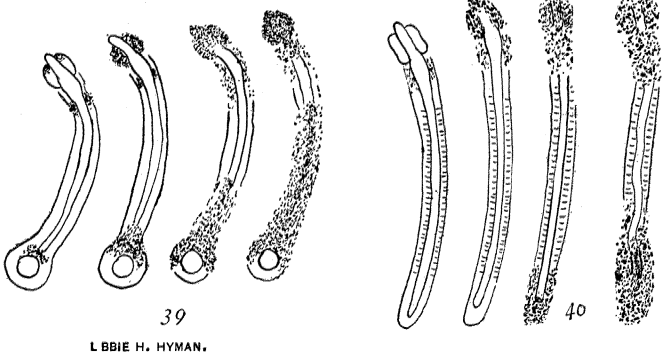

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